Biomimetic Conditioning of Human Dentin Using Citric Acid

Ivica, Anja ; Zehnder, Matthias ; Mateos, José M ; Ghayor, Chafik ; Weber, Franz E

Abstract: INTRODUCTION In carious teeth, transforming growth factor beta 1 (TGF- 1) is released from the dentin matrix and possibly activated in an acidic environment. Conversely, EDTA solutions with a neutral to slightly alkaline pH are used in clinics to promote cell homing in regenerative endodontic procedures. We hypothesized that citric acid (CA) might be more beneficial. METHODS TGF- 1 release from human dentin disks conditioned with either 10% CA (pH = 2) or 17% EDTA (pH = 8) and the behavior of human stem cells toward such pretreated dentin were studied. The protein concentration in conditioning solutions after 10 minutes of dentin exposure was determined using a pH-independent slot blot technique. RESULTS There was a 5-fold higher concentration of the target protein in CA (382 ± 30 ng/disk) compared with EDTA (66 ± 3 ng/disk, $P < .005$). Using confocal laser scanning microscopy on immunofluorescent-labeled disks, we identified a high density of TGF- 1 in peritubular dentin after CA treatment. A migration assay showed that CA conditioning attracted significantly more stem cells toward the dentin after 24 hours compared with EDTA ($P < .05$) or phosphate-buffered saline ($P < .005$). To investigate whether the cell response to these dentin surfaces could be affected by different pretreatments, we cultured stem cells on conditioned dentin disks and found that CA had a significantly ($P < .05$) better effect than EDTA on cell attachment and cell survival. CONCLUSIONS CA conditioning could be useful and may have significant benefits over current treatments.

DOI: https://doi.org/10.1016/j.joen.2018.09.015

Biomimetic Conditioning of Human Dentin Using Citric Acid

Anja Ivica, DDS, Matthias Zehnder, DDS, PhD,† José M. Mateos, PhD,‡ Chafik Ghayor, PhD,* and Franz E. Weber, PhD**

Abstract

Introduction: In carious teeth, transforming growth factor beta 1 (TGF-β1) is released from the dentin matrix and possibly activated in an acidic environment. Conversely, EDTA solutions with a neutral to slightly alkaline pH are used in clinics to promote cell homing in regenerative endodontic procedures. We hypothesized that citric acid (CA) might be more beneficial. **Methods:** TGF-β1 release from human dentin disks conditioned with either 10% CA (pH = 2) or 17% EDTA (pH = 8) and the behavior of human stem cells toward such pretreated dentin were studied. The protein concentration in conditioning solutions after 10 minutes of dentin exposure was determined using a pH-independent slot blot technique. **Results:** There was a 5-fold higher concentration of the target protein in CA (382 $\pm$ 30 ng/disk) compared with EDTA (66 $\pm$ 3 ng/disk, $P < .005$). Using confocal laser scanning microscopy on immunofluorescent-labeled disks, we identified a high density of TGF-β1 in peritubular dentin after CA treatment. A migration assay showed that CA conditioning attracted significantly more stem cells toward the dentin after 24 hours compared with EDTA ($P < .05$) or phosphate-buffered saline ($P < .005$). To investigate whether the cell response to these dentin surfaces could be affected by different pretreatments, we cultured stem cells on conditioned dentin disks and found that CA had a significantly ($P < .05$) better effect than EDTA on cell attachment and cell survival. **Conclusions:** CA conditioning could be useful and may have significant benefits over current treatments. *(J Endod 2018;■:1–6)*

Key Words

Bioengineering, cell-matrix interactions, dentin, growth factor(s), regeneration

Significance

The results of the current study suggest the possibility that a 10% citric acid solution could be more beneficial than the gold standard 17% EDTA solution for cell homing in regenerative endodontic procedures.

A long-standing aspiration in dentistry has been to regenerate a pulp (1). To this end, the currently favored clinical approach is a strategy called cell homing (2, 3). The procedure involves disinfection and deproteinization using a sodium hypochlorite (NaOCl) solution followed by conditioning with EDTA, controlled bleeding, and tooth restoration (4). The concept is currently limited to immature teeth (5). However, it is conceivable that the procedure could one day become applicable to all teeth affected by apical periodontitis.

Chemically released growth factors are important in regenerative procedures (6, 7). Transforming growth factor beta 1 (TGF-β1) induces cell proliferation, differentiation, and chemotaxis in different cell types. Hence, it is considered the key molecule for pulp regeneration (8). The dentin matrix acts as a reservoir from which growth factors can be released on demand. In nature, this occurs (eg, in an acidic environment during development of caries to promote tertiary dentinogenesis underneath the site of attack) (9). The release of growth factors can also be induced during chemomechanical root canal treatment by removing the inorganic portion of the smear layer and conditioning the canal wall (10). The most common agent used for this step during regenerative procedures is an EDTA solution, usually at a concentration of 17% and a pH of 8 (11). EDTA does not dissolve in an acidic environment, and its solubility increases with pH (12). Citric acid (CA) solutions in the 10% range are also commonly used for standard endodontic procedures (13). However, in the context of regenerative endodontics, it had been claimed that TGF-β1 could be released by EDTA and not by CA (14). It should be noted that the enzyme-linked immunosorbent assay method that was used to gain these results is pH sensitive (15). Moreover, it has been shown that very low and very high pH levels (1.5 or 12) help to convert the inactive form of TGF-β into its active form (16). Consequently, it may be speculated that for pulp regeneration CA (with a pH around 2) could be more advantageous than a disodium EDTA solution, which has a neutral or slightly alkaline pH.

Two distinct factors seem to play a role in regenerative endodontic procedures: the release of molecular cues from dentin and the exposure of these same cues (17). Hence, the goals of the present study were 2-fold:

1. To check the release of TGF-β1 from human dentin treated with either CA or EDTA using a pH-insensitive method and to assess the induction of cell migration

From the Departments of *Cranio-Maxillofacial and Oral Surgery, Oral Biotechnology and Bioengineering; †Preventive Dentistry, Periodontology and Cariology; and ‡Center for Microscopy and Image Analysis, University of Zurich, Zurich, Switzerland.

Address requests for reprints to Dr Franz E. Weber, University of Zurich, Center for Dental Medicine/Cranio-Maxillofacial and Oral Surgery, Oral Biotechnology and Bioengineering, Plattenstrasse 11, CH-8032, Zürich, Switzerland. E-mail address: franz.weber@zzm.uzh.ch

0099-2399

https://doi.org/10.1016/j.joen.2018.09.015

2. To visualize TGF-β1 on dentin by immunofluorescence confocal laser scanning microscopy and to assess cell–pretreated dentin interaction

We speculated that CA should be more potent than EDTA to release and expose TGF-β1 from/on dentin and should thus have a better effect on cell attraction, attachment, and survival.

Materials and Methods

Preparation of Dentin Disks

For this study, we used extracted human molars from the department's collection. All patients had given informed consent. The teeth were stored in tap water and cut with a circular saw (Leica, Wetzlar, Germany) under water cooling. The disks (200-μm thick) were prepared from below the cementoenamel junction. Three adjacent disks were cut from the same tooth and randomly allocated to the treatment group. For each experiment, we used at least 3 different teeth. To check if the groups were well-balanced, the disk diameter was measured using a MarCal caliper (Mahr GmbH, Esslingen, Germany), and the dentin surface was calculated using ImageJ software (National Institutes of Health, Bethesda, MD).

The following solutions (300 μL) were used as conditioning agents: 10% CA (pH = 2, 476 mol/L), 17% Na_2 EDTA (adjusted to a pH of 8), or phosphate-buffered saline (PBS; Gibco, Paisley, UK). The conditioning treatment was 10 minutes.

Slot Blot Protein Immunoassay for TGF-β1 Release

The protein concentration after conditioning was determined using a slot blot technique. The probes were directly bound to a nitrocellulose membrane using a Bio-Dot apparatus (Bio Rad, Hercules, CA). As a standard, recombinant human TGF-β1 (Sigma-Aldrich, St Louis, MO) in concentrations from 0.2–12.5 ng/μL was loaded. The membrane was blocked with 3% gelatin (Sigma-Aldrich) in Tris-buffered saline. Samples were incubated with anti–TGF-β1 produced in mouse (Sigma-Aldrich) and horse/antimouse immunoglobulin G and horseradish peroxidase–linked antibody (Cell Signaling Technology, Danvers, MA). Intensities of bands were analyzed using Image Lab software (BioRad). A precision balance (PJ3000; Mettler Toledo, Nänikon, Switzerland) was used to weigh disks before the experiment.

Spatial Distribution of TGF-β1 on CA Pretreated Dentin

In order to investigate the expression of TGF-β1 on the pretreated surface, an immunofluorescence study was performed. CA pretreated disks were washed and blocked with 0.2% gelatin and 0.5% bovine serum albumin (Albumin Fraction V; AppliChem, Darmstadt, Germany) in PBS. Samples were incubated with chicken anti–TGF-β1 (Invitrogen, Carlsbad, CA) 1:30 and goat antichicken Alexa Fluor 568 (Invitrogen) 1:200. Samples were observed with confocal laser scanning microscopy (SP8 Inverse STED 3X, Leica) and excited with a white light laser (561 nm). Images were acquired with a 63×/1.4 numerical aperture oil objective. A hybrid detector (564–651 nm) was used for fluorescence and a photomultiplier tube for transmission detection. For negative controls, disks were incubated only with secondary antibody or both antibodies were withdrawn.

Cell Culture

Human bone marrow–derived mesenchymal stem cells (PCS-500-012; American Type Culture Collection, Manassas, VA) were cultured in MesenPro RS with 1% Pen Strep and 1% GlutaMAX-I (Gibco). Cells at passages 3–6 at 80% confluence were used in all experiments.

Cell Migration

To test cell migration, polycarbonate Transwell inserts with a pore size of 8 μm (Corning, Corning, NY) were used. Cells were starved overnight and added to inserts. Dentin disks were disinfected with 5% NaOCl and randomly allocated to the groups. Disks were placed on the bottom of the well together with 650 μL starvation medium. Inserts preseeded with cells were immerged into the wells and maintained in the incubator for 24 hours. After fixation in 3.7% formaldehyde, cells were permeabilized by 100% methanol and stained with crystal violet (Acros Organics, Geel, Belgium). The inside of each insert was cleaned using cotton swabs. Cells, migrated from the inside of the insert to the other side of the membrane facing the dentin slices, were visualized using an inverted microscope (CKX53; Olympus, Tokyo, Japan). Ten fields were randomly selected from each insert, and cells were counted therein.

Cell Attachment

Dentin disks were disinfected with 5% NaOCl and randomly divided into conditioning groups. After washing, 6 disks from the same group were placed in a single layer in a petri dish. On the top of every disk, cells at a density of 5.5×10^5 cells/mL were seeded. The petri dish was kept in the incubator overnight. The next day disks were placed in a new petri dish, washed, detached, and counted using an EVE Automatic Cell Counter (NanoEnTek, Seoul, Korea).

Cell Survival

Everything before the incubation step was performed in the same manner as in the cell attachment assay described previously. The petri dish was kept in the incubator for 48 hours. After trypsinization, cells were counted.

Statistical Analysis

All experiments were repeated at least 3 times independently. Data were statistically analyzed using Prism 7 software (GraphPad, La Jolla, CA). Results are expressed as mean values ± standard deviation and were compared by 1-way analysis of variance and the Student *t* test. Bonferroni adjustment was applied for multiple comparisons. The level of statistical significance was set at $P < .05$.

Results

Slot Blot Protein Immunoassay for TGF-β1 Release

The discrete band was visible when a standard at a concentration of 0.4 ng/μL was loaded, which corresponded to 117 ng recombinant TGF-β1, whereas saturation occurred at 3750 ng (Fig. 1*A*). PBS treatment showed no staining on the membrane, neither did pure CA or EDTA. A significantly ($P < .05$) higher release of the target protein was evident in the CA group (Fig. 1*B*), with 382 ± 30 ng TGF-β1 per dentin disk, compared with 66 ± 3 ng TGF-β1 released with EDTA.

Spatial Distribution of TGF-β1 on CA Pretreated Dentin

A high amount of TGF-β1 was exposed and detectable in the CA group (Fig. 2*A–C*). The exposed protein formed roundish structures. By overlaying with a bright-field view, we perceived that a high exposure of the protein was manifested in peritubular dentin. On the dentin disks without antibody incubation, the protein was not detected (Fig. 2*D–F*).

Cell Migration

The modified Boyden chamber assay showed that both CA and EDTA conditioning induced migration of stem cells toward pretreated dentin (Fig. 3*A–D*) but CA treatment significantly more than EDTA ($P < .05$) or PBS ($P < .01$).

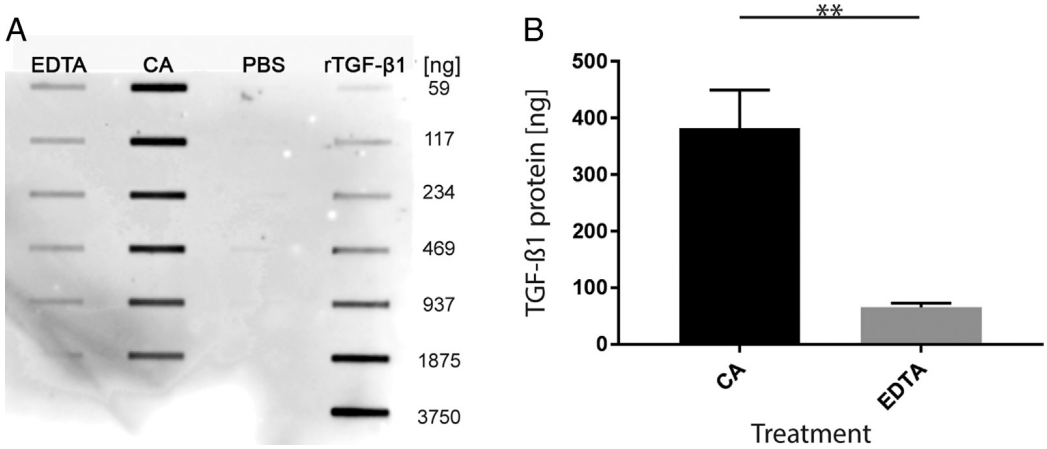

Figure 1. Quantification of TGF-β1 released by conditioning solutions. (*A*) According to band intensities on the membrane, we calculated the (*B*) amount of released TGF-β1 per disk. In the treatment groups, after conditioning 3 disks with the same agent, solutions were mixed and loaded to the membrane in duplicates. The same procedure was repeated 3 times ($n = 3$). The membrane did not show a signal for the wells filled with pure EDTA, CA (last row in the treatment groups), or PBS. The last column was loaded with a 2-fold dilution series from 3750 ng recombinant TGF-β1 down to 59 ng. CA proved to be the more effective agent for releasing TGF-β1 compared with EDTA (**$P < .01$). The error bars indicate standard deviation.

Cell Attachment and Survival

The cell-dentin interaction was investigated using cell attachment (Fig. 4*A*) and cell survival assays (Fig. 4*B*). The attachment was significantly more promoted by CA than EDTA ($P < .05$) or PBS ($P < .01$). Moreover, cell survival was also significantly ($P < .05$) higher after CA treatment compared with EDTA or PBS.

Discussion

The humoral defense against caries and recruitment of pluripotent cells to replace damaged odontoblasts occurs in an acidic environment. This study showed that 10% CA released and exposed more TGF-β1 from human root dentin than 17% EDTA did. Consequently, more

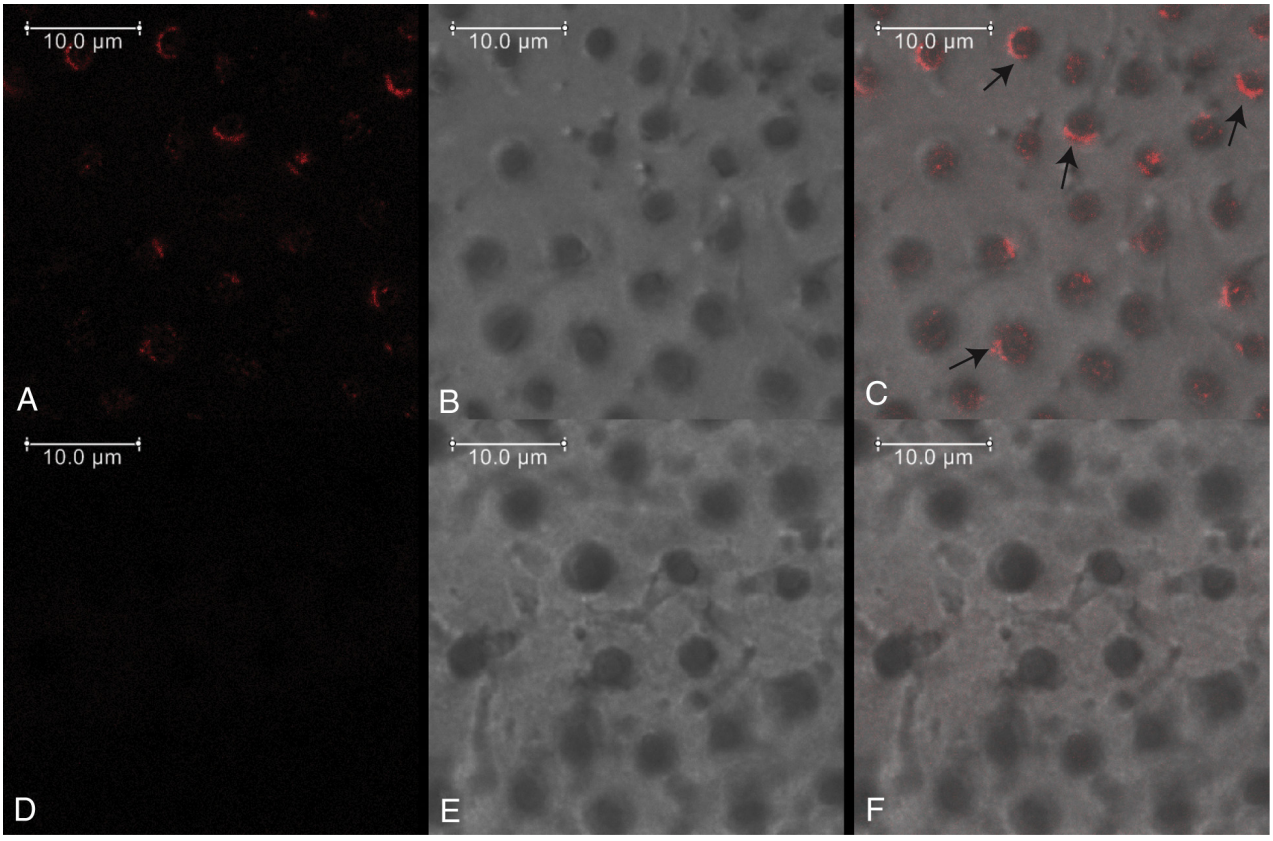

Figure 2. TGF-β1 exposure upon conditioning of the dentin surface. Spatial distribution of TGF-β1 was assessed using confocal microscopy. (*A* and *D*) Fluorescence, (*B* and *E*) bright-field, and (*C* and *F*) overlay images. (*A–C*) Orthogonal sections of tubules on a dentin disk treated with CA showed exposure of TGF-β1 around the open tubules (*arrows*), whereas the negative control did not show any detectable signal. The scale bar represents 10 μm.

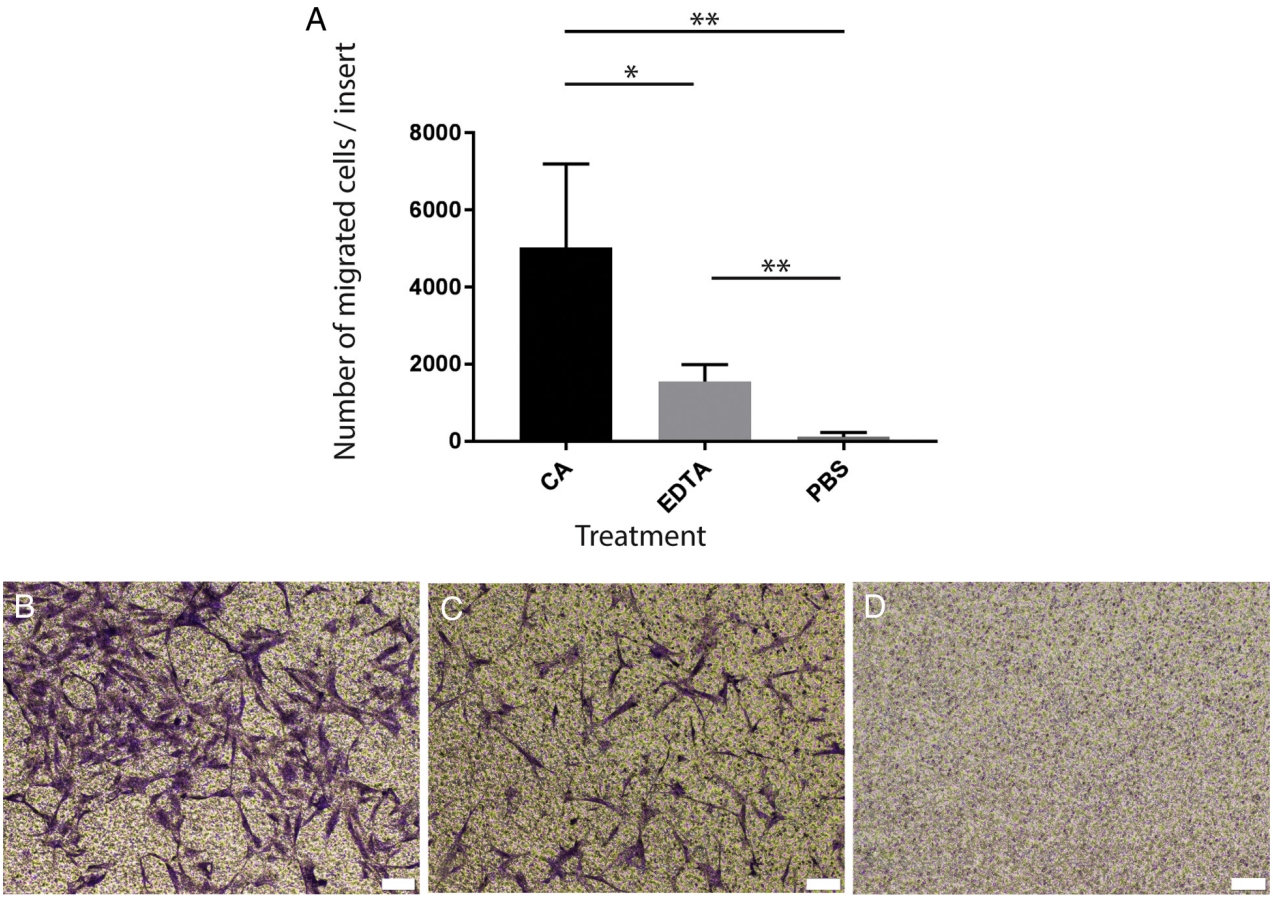

Figure 3. Cell migration toward dentin disks 24 hours after the cells were juxtaposed to test and control the dentin disks in the lower chamber of the incubation plates. The total number of migrated cells per insert is shown in panel *A*. Representative images of the (*B*) bottom of Transwell inserts showing stem cells migrated toward CA and (*C*) an EDTA-pretreated disk showing that CA conditioning attracted significantly more stem cells compared with EDTA ($P < .05$, $n = 4$). (*D*) Treatment with PBS did not cause notable migration. The scale bar represents 100 μm. The error bars indicate standard deviation. The difference between all groups was tested by 1-way analysis of variance/Bonferroni adjustment (*$P < .05$, **$P < .01$).

pluripotent cells were attracted and adhered to such biomimetically conditioned dentin surfaces.

The current data were obtained in the laboratory using human specimens. Consequently, no clinical conclusions should be drawn. However, there was a similarity to dental clinics. For disinfection, we used NaOCl, which is the most common disinfectant in regenerative endodontics (11). CA and EDTA are commonly used as demineralizing agents. Until now, EDTA has been considered as the gold standard for

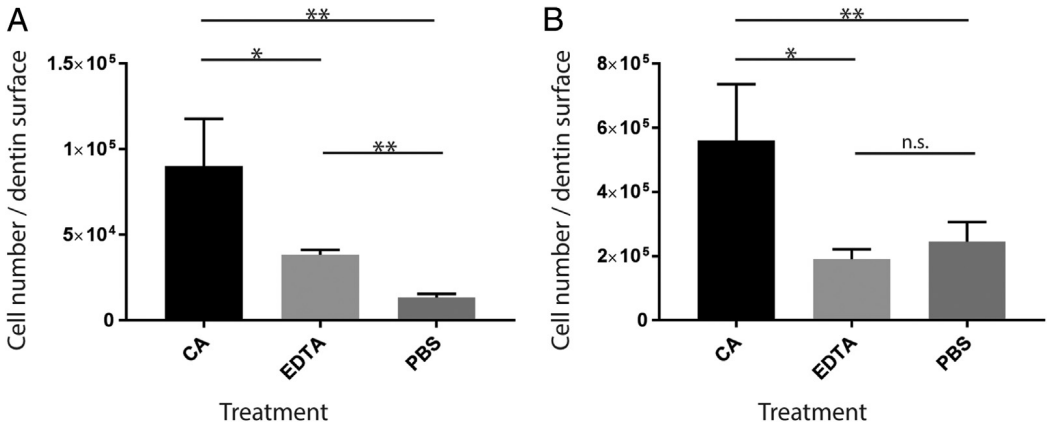

Figure 4. Stem cell interaction with dentin surfaces upon conditioning. Stem cells were seeded on differently conditioned dentin slices. (*A*) After 24 hours, cells that attached to the dentin surface were counted, (*B*) whereas after 48 hours the whole number of live cells on each dentin disk was measured. Statistical differences exist between groups (analysis of variance, $P < .01$). CA promoted attachment ($n = 3$) and survival ($n = 4$) of stem cells compared with EDTA treatment (*$P < .05$) or PBS treatment (**$P < .01$). A nonsignificant difference (n.s.) was observed between survivals after EDTA versus PBS treatment. The error bars represent standard deviation.

regenerative endodontic procedures (18). Furthermore, we applied human bone marrow–derived mesenchymal stem cells because they represent 1 of the main sources of stem cells in the oral cavity (19). Dentin specimens were exposed to test and control irrigants for 10 minutes, which appears to be clinically relevant (14).

The main problem after the cell homing strategy is to recruit the patient's endogenous mesenchymal stem cells (20, 21). This must be followed by cell attachment, differentiation toward odontoblasts, and integration into normal tissue (22–24). Our results differ to a certain extent from those obtained by Zhao et al (25), who found a higher density of apparently exposed TGF-β1 on specimens treated with EDTA compared with CA. However, they also observed better smear layer removal with EDTA compared with CA, which is in contrast to the literature that specifically dealt with this topic. Because dentin is a rather heterogeneous tissue, results obtained by mere 2-dimensional imaging should be interpreted with care (26). Others applied ELISA to quantify TGF-β1 extraction from dentin (14, 18, 22), but no significant level of TGF-β1 was detected for CA treatment. This is in contrast to our results clearly showing a significant TGF-β1 release upon CA treatment and the presence of TGF-β1 protein around tubular openings. Moreover, our results of spatial distribution of TGF-β1 on dentin slices indicate that TGF-β1 is exposed in peritubular dentin, which is known to contain an organic matrix, including bioactive macromolecules (27). The apparent underestimation of TGF-β1 in case of CA conditioning by other authors could have been caused by the susceptibility of the ELISA method to acidic conditions (15). Indeed, protonation causes changes on the amino acids and dissociation of binding proteins that may compete for the same binding spots with ELISA antibodies and block the signal from the targeted protein (28). To overcome these possible pH effects, we applied a slot blot assay known to be a suitable alternative to ELISA. The method applied here is highly reliable, inexpensive, and repeatable (29, 30). This higher level of TGF-β1 release by CA conditioning is also in line with the increased migration toward CA conditioned dentin compared with EDTA treatment (Fig. 3).

It is well-known that increasing the roughness of the surface improves cell attachment and growth (31, 32). A study on root canals showed that conditioning with CA significantly increased the roughness of the dentin compared with EDTA treatment (33). Scanning electron microscopy showed a higher number and a wider diameter of open dentinal tubules after CA treatment (34). These reports are in line with our findings (Figs. 3 and 4); the increased roughness upon CA treatment could be 1 of the reasons for the superiority of CA conditioning in terms of stem cell attachment and survival. Moreover, acidification converts latent TGF-β to its active form (8, 16) suggesting that an acidic environment during caries may play a natural role in the regulation of TGF-β activation.

TGF-β1 is known to induce cell proliferation, differentiation, and chemotaxis in different cell types (8). However, other growth may contribute to the observed regenerative potential. It has been shown that CA treatment was more potent to extract bone morphogenetic protein 2 and vascular endothelial growth factor from dentin slices than EDTA treatment (18). In regenerative endodontics, TGF-β1 was found to promote the migration of dental pulp stem cells (24). This capability depends on the dose of TGF-β1 and the cell type used in the study (7, 22, 35). This was confirmed using human stem cells in this investigation. However, in our study, CA induced a 3-fold higher cell number than EDTA to migrate toward the conditioned dentin.

In conclusion, this study provided direct evidence of the release and exposure of TGF-β1 from and on chemically conditioned human dentin. CA treatment appeared superior to the gold standard EDTA in terms of TGF-β1 release as well as stem cell recruitment, attachment, and survival. Therefore, CA conditioning, which mimics natural conditions under caries attack, may improve pulp regeneration.

Acknowledgments

We thank Andres Kaech for scientific comments; Gery Barmettler for technical support with sample preparation of the immunofluorescence experiments; Claudia Cucuzza and Beatrice Sener for swift laboratory work; and Tse-Hsiang Chen, Nupur Khera, Indranil Bhattacharya, Kathrin Schumann, and Ana Perez for their helpful advice.

Supported by a Swiss Government Excellence Scholarship (A.I.).

The authors deny any conflicts of interest related to this study.

References

1. Nygaard-Østby B. The role of the blood clot in endodontic therapy. An experimental histologic study. Acta Odontol Scand 1961;19:324–53.
2. Lee CH, Cook JL, Mendelson A, et al. Regeneration of the articular surface of the rabbit synovial joint by cell homing: a proof of concept study. Lancet 2010;376:440–8.
3. Kim JY, Xin X, Moioli EK, et al. Regeneration of dental-pulp-like tissue by chemotaxis-induced cell homing. Tissue Eng Part A 2010;16:3023–31.
4. He L, Zhong J, Gong Q, et al. Regenerative endodontics by cell homing. Dent Clin North Am 2017;61:143–59.
5. Fang Y, Wang X, Zhu J, et al. Influence of apical diameter on the outcome of regenerative endodontic treatment in teeth with pulp necrosis: a review. J Endod 2018;44:414–31.
6. Kaushik SN, Kim B, Walma AM, et al. Biomimetic microenvironments for regenerative endodontics. Biomater Res 2016;20:14.
7. Galler KM, Widbiller M, Buchalla W, et al. EDTA conditioning of dentine promotes adhesion, migration and differentiation of dental pulp stem cells. Int Endod J 2016;49:581–90.
8. Niwa T, Yamakoshi Y, Yamazaki H, et al. The dynamics of TGF-beta in dental pulp, odontoblasts and dentin. Sci Rep 2018;8:4450.
9. Magloire H, Romeas A, Melin M, et al. Molecular regulation of odontoblast activity under dentin injury. Adv Dent Res 2001;15:46–50.
10. Zehnder M. Root canal irrigants. J Endod 2006;32:389–98.
11. Diogenes AR, Ruparel NB, Teixeira FB, Hargreaves KM. Translational science in disinfection for regenerative endodontics. J Endod 2014;40(Suppl):S52–7.
12. Biel P, Mohn D, Attin T, Zehnder M. Interactions between the tetrasodium salts of EDTA and 1-hydroxyethane 1,1-diphosphonic acid with sodium hypochlorite irrigants. J Endod 2017;43:657–61.
13. Loel DA. Use of acid cleanser in endodontic therapy. J Am Dent Assoc 1975;90:148–51.
14. Galler KM, Buchalla W, Hiller KA, et al. Influence of root canal disinfectants on growth factor release from dentin. J Endod 2015;41:363–8.
15. Doucet J, Zhao A, Fu J, Avrameas A. Development and validation of an ELISA at acidic pH for the quantitative determination of IL-13 in human plasma and serum. Dis Markers 2013;35:465–74.
16. Lyons RM, Keski-Oja J, Moses HL. Proteolytic activation of latent transforming growth factor-beta from fibroblast-conditioned medium. J Cell Biol 1988;106:1659–65.
17. Smith AJ, Scheven BA, Takahashi Y, et al. Dentine as a bioactive extracellular matrix. Arch Oral Biol 2012;57:109–21.
18. Sadaghiani L, Gleeson HB, Youde S, et al. Growth factor liberation and DPSC response following dentine conditioning. J Dent Res 2016;95:1298–307.
19. Egusa H, Sonoyama W, Nishimura M, et al. Stem cells in dentistry–part I: stem cell sources. J Prosthodont Res 2012;56:151–65.
20. AAE. *Scope of Endodontics: Regenerative Endodontics*. Available at: https://www.aae.org/specialty/wp-content/uploads/sites/2/2017/06/scopeofendo_regendo.pdf. Accessed April 10, 2018.
21. Galler KM. Clinical procedures for revitalization: current knowledge and considerations. Int Endod J 2016;49:926–36.
22. Zeng Q, Nguyen S, Zhang H, et al. Release of growth factors into root canal by irrigations in regenerative endodontics. J Endod 2016;42:1760–6.
23. Ruangsawasdi N, Zehnder M, Patcas R, et al. Effects of stem cell factor on cell homing during functional pulp regeneration in human immature teeth. Tissue Eng Part A 2017;23:115–23.
24. Howard C, Murray PE, Namerow KN. Dental pulp stem cell migration. J Endod 2010;36:1963–6.

25. Zhao S, Sloan AJ, Murray PE, et al. Ultrastructural localisation of TGF-beta exposure in dentine by chemical treatment. Histochem J 2000;32:489–94.
26. De-Deus G, Reis C, Di Giorgi K, et al. Interfacial adaptation of the Epiphany self-adhesive sealer to root dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;111:381–6.
27. Bertassoni LE, Stankoska K, Swain MV. Insights into the structure and composition of the peritubular dentin organic matrix and the lamina limitans. Micron 2012;43:229–36.
28. Lakshman KM, Bhasin S, Corcoran C, et al. Measurement of myostatin concentrations in human serum: circulating concentrations in young and older men and effects of testosterone administration. Mol Cell Endocrinol 2009;302:26–32.
29. Stone W, Grabias B, Lanke K, et al. A comparison of Plasmodium falciparum circumsporozoite protein-based slot blot and ELISA immuno-assays for oocyst detection in mosquito homogenates. Malar J 2015;14:451.
30. Li C, Jaentschke B, Song Y, et al. A simple slot blot for the detection of virtually all subtypes of the influenza A viral hemagglutinins using universal antibodies targeting the fusion peptide. Nat Protoc 2010;5:14–9.
31. Stevens MM, George JH. Exploring and engineering the cell surface interface. Science 2005;310:1135–8.
32. Matthews JA, Wnek GE, Simpson DG, Bowlin GL. Electrospinning of collagen nanofibers. Biomacromolecules 2002;3:232–8.
33. Eldeniz AU, Erdemir A, Belli S. Effect of EDTA and citric acid solutions on the microhardness and the roughness of human root canal dentin. J Endod 2005;31:107–10.
34. Reis C, De-Deus G, Leal F, et al. Strong effect on dentin after the use of high concentrations of citric acid: an assessment with co-site optical microscopy and ESEM. Dent Mater 2008;24:1608–15.
35. Goncalves LF, Fernandes AP, Cosme-Silva L, et al. Effect of EDTA on TGF-beta1 released from the dentin matrix and its influence on dental pulp stem cell migration. Brazil Oral Res 2016;30:e131.